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DESIGN AND DEVELOPMENT OF NANOSCALE BIOMOTOR POWER UNITS

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6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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AFOSR/NL
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13. SUPPLEMENTARY NOTES

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14. ABSTRACT

1. We have continued to refine our theoretical model for the design of a bacterial cell powered motor. 2. We have determined what types of surfaces bind motile bacterial cells. 3. We have monitored surface adhered bacterial cell motility using fluorescent dyes and found that cells remain alive and motile for more than 4 hours. 4. We have discovered that E. coli bacterial cells will not bind to surface dot features with a diameter of 1.2 um or smaller. 5. We have designed and fabricated "holed" surfaces that bind motile bacterial cells in a "nose-on" fashion. 6. We have used DPN to attach bacterial cells to surfaces. 7. We have obtained and attached CheY deficient (Pseudomonas aeruginosa) "smooth swimming" bacterial cells to prefabricated micro-array surfaces. 8. We have generated an initial design and fabricated a prototype micro-scale biomotor. 9. We have "proof-of-concept" that motile bacterial cells can spin a device. Progress in the last four years has been excellent, and included the publication of three manuscript (Small, Talanta and, Langmuir). We currently have at least two additional manuscripts in preparation.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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2. STATUS OF WORK (LIMIT one paragraph)

A brief statement of progress towards achieving the research objectives. Insert lines as needed.

Progress in the last four years has been excellent, and included the publication of three manuscripts (*Small, Talanta and, Langmuir).* We currently have at least two additional manuscripts in preparation. The PI has given 13 invited seminars in the past four years related to this project and have presented 3 posters. The following objectives have been accomplished during the past four years:

1. We have continued to refine our theoretical model for the design of a bacterial cell powered motor.
2. We have determined what types of surfaces bind motile bacterial cells.
3. We have monitored surface adhered bacterial cell motility using fluorescent dyes and found that cells remain alive and motile for more than 4 hours.
4. We have discovered that *E. coli* bacterial cells will not bind to surface dot features with a diameter of 1.2 μm or smaller
5. We have designed and fabricated "holed" surfaces that bind motile bacterial cells in a "nose-on" fashion.
6. We have used DPN to attach bacterial cells to surfaces.
7. We have obtained and attached CheY deficient (*Pseudomonas aeruginosa*) "smooth swimming" bacterial cells to prefabricated micro-array surfaces.
8. We have generated an initial design and fabricated a prototype micro-scale biomotor.
9. We have "proof-of-concept" that motile bacterial cells can spin a device.

3. ACCOMPLISHMENTS/NEW FINDINGS (LIMIT approx. one page)

Describe research highlights, their significance to the field, their relationship to the original goals, their relevance to the AF's mission, and their potential applications to AF and civilian technology challenges. Insert lines as needed.

During the first two years of this grant, several important discoveries were made with regard to how to attach motile bacterial cells to surfaces in specific, pre-designed microarrays. First, we developed a method to fabricate microarrays capable of binding motile *Escherichia coli* bacterial cells using 16-mercaptohexadecanoic acid (MHA)-patterned microarrays, that were covalently functionalized with *E. coli* antibodies, lipopolysaccharide, or poly-L-lysine (PLL). By utilizing 11-mercaptoundecyl-penta(ethylene glycol) (PEG-SH) or 11-mercapto-1-undecanol (MOU) as passivity molecules, non-specific binding of *E. coli* was nearly completely inhibited. Microcontact printing was used to prepare microarrays for bacterial cell adhesion, and bacterial attachment was studied by optical/fluorescence and atomic force microscopy (AFM). Our data indicate that a single motile *E. coli* bacterial cell can be attached to pre-designed line and/or dot features and binding can occur *via* the cell body or the bacterium's flagellum.

Second, we have shown that *E. coli* K-12 bacterial cells are alive after adhesion onto pre-fabricated surface structures for more than 4 hours based on direct observations with an optical microscope. We have verified these data by examining patterned *E. coli* cells that were treated with two nucleic acid stains, green-fluorescent SYTO-9 and red-fluorescent propidium iodide. Since both live and dead bacteria can be viewed directly, our data indicate that the majority (> 90 %) of the surface patterned cells are viable when captured on the protein microarrays for more

than 4 hours. These data indicate that surface adhered bacterial cells remain motile for extended periods of time, thus providing "proof-of-concept" that surface bound cells can be used to power a biomotor.

Third, we have determined the minimum surface feature size that will still bind an *E. coli* K-12 bacterial cell using a size-variable (1.0 to 3.0 μm via 0.1 μm steps) PLL-MHA dot microarray. These data indicated that the minimum surface feature size that effectively binds *E. coli* must be at least 1.3 μm. Other bacterial cell types may prefer different surface feature sizes and this aspect is currently under investigation. The motility of adsorbed bacterial cells on the size-variable PLL-MHA dot array was monitored with an optical microscope and the surface adhered *E. coli* cells remained alive and motile for more than 4 hours.

Fourth, we have prepared a substrate that contains a series of "holes" *via* E-beam lithography, in order to dictate the way in which motile bacterial cells bind to surfaces, *i.e.* in a "nose-on" fashion. Our initial surfaces contain holes that are 3 μm x 0.5 μm in which the gold surface at the bottom of the holes have been coated with MHA followed by PLL. The regions between holes have been passivated with PEG. These surfaces show great promise for the attachment of motile bacteria in a "nose-on" fashion. We have been able to bind single motile bacterial cells in a hole and have seen some specificity in the orientation of bacterial cell adhesion.

Fifth, we have also observed pH dependence to bacterial cell adhesion in which nearly all of the surface features contain bacterial cells at pH 9 while nearly all are empty at pH 4.0. This is likely the result of both destroying the MHA-PLL surface as well as cellular surface charges. In addition, we have used electrochemical methods to bind motile bacterial cells to surfaces.

Sixth, we have attached the CheY deficient *P. aeruginosa* "smooth-swimming" bacterial cells to micro-array surfaces. *P. aeruginosa* represent the bacterial cell of choice to power a micron scale biomotor. *P. aeruginosa* are monotricus bacterial cells that swim much more vigorously than *E. coli* (~40% faster). During the past year we have obtained the CheY deficient *P. aeruginosa* cell line, which is a "smooth-swimming" bacterial cell line, so it does not undergo chemotaxis. We have utilized our previously developed attachment methodologies, which involve patterning 16-mercaptohexadecanoic acid (MHA) micro-arrays that were covalently functionalized with poly-L-lysine (PLL). By utilizing 11-mercapto-1 undecanol (MOU) as a passivative molecule, nonspecific binding of *P. aeruginosa* was nearly completely inhibited.

Seventh, Dip-pen nanolithography (DPN) was also used to prepare microarrays for bacterial cell adhesion. DPN is a particularly important soft-lithographic method since it allows us to place whole bacterial cells on a surface in predesigned, site-specific micro-arrays. We examined *P. aeruginosa* attachment to DPN generated micro-arrays *via* optical and atomic force microscopy (AFM). Our data indicate that a single motile *P. aeruginosa* bacterial cell can be attached to predesigned line and/or dot features and binding occurs *via* the cell body or the bacterium's flagellum. These cells are alive upon adhesion to the DPN generated micro-array for more than four hours based on direct observations with an optical microscopy.

Finally, we have designed, fabricated and tested a biomotor device, in solution, that is spun by *P. aeruginosa* motile bacterial cells (Figure 1).

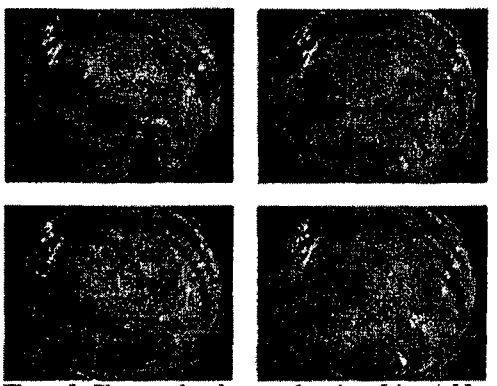

Figure 1. Photographs taken as a function of time (~15 sec intervals) showing that bacterial cells can spin a device suspended in solution.

In this device, *P. aeruginosa* cells are attached to a glass slide cover-slip in which one-half of each side is coated with gold and suspended *via* a 25 mm gold thread in DI water. The sample on the lower left hand side is rotating clockwise, the one on the upper right side is rotating counterclockwise whereas the sample on the lower right, which is a control sample that does not have bacteria bound to it, is not rotating at all. We used asymmetry to spin the two slides in opposite directions in order to rule out movement due to fluid motion. When these samples are removed from solution and allowed to dry, thereby killing the bacterial cells, none of the devices spin. These data indicate that mass imbalance is not causing the observed device spinning. In all of our experiments done to date, the only time we see a device spin is when bacteria are attached and we can control the direction of device spinning based on where we attach the bacterial cells. Moreover, these devices can spin for up to 2 min., after which tension builds up in the 25 mm gold thread, which is greater than then power generated by the swimming bacterial cells. Patterning cells on these devices provides an increase in the speed of rotation by a factor of 3, verifying our theoretical predictions. In order to verify that bacterial cells are in fact attached to the gold covered portions of the devices and not the glass sections, optical and LFM images were collected of the attached bacterial cells (Figure 2). Optical and LFM images indicate that bacterial cells are attached only to the gold-covered surface with little or no binding to bare silicon surfaces. Combination of these data, provide the first evidence that a biomotor powered by motile bacterial cells is an attainable goal.

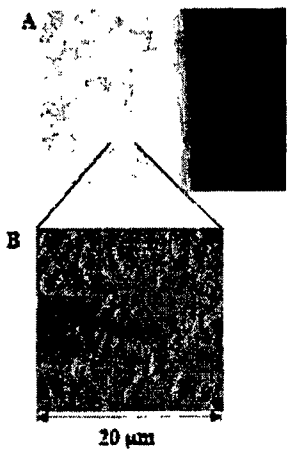

Figure 2. A) Optical image of the gold/glass interface and B) LFM image of attached bacterial cells.

4. PERSONNEL SUPPORTED

Postdocs	Percentage of Salary Provided by Grant
Dr. Sergey Rozhok	100%
Dr. Sabina Swierczek	25%
Dr. Dorjderem Nyamjav	100%

Graduate Students:	Percentage of Salary Provided by Grant
Vincent Zachary	100 %

Undergraduate Students: Percentage of Salary Provided by Grant
None

Visiting Faculty/Scientists: Percentage of Salary Provided by Grant
None

5. PUBLICATIONS

Peer-reviewed publications submitted and/or accepted during the 12-month period. Insert lines below as needed.

A. Journal Articles

1. Rozhok, Sergey; Kwang-Fu Shen, Clifton; Littler, Pey-Lih Ho; Fan, Zhifang; Liu, Chang; Mirkin*, Chad A.; Holz*, Richard C. "Methods for Fabricating Microarrays of Motile Bacteria" *Small*, **2005**, *1*, 445-451.
2. Rozhok, Sergey; Holz*, Richard C. "Electrochemical Adhesion of Motile Bacteria to Gold" *Tantala*, **2005**, *66*, 538-542.
3. Rozhok, Sergey; Fan, Zhifang; Liu, Chang; Mirkin, Chad A.; Holz*, Richard C. "Methods for Fabricating Microarrays of Motile Bacteria: Controlling Bacterial Cell Orientation" *Langmuir.*, **2006**, *22*, 11251-11254.
4. Nyamjav, Dorjderem; Rozhok, Sergey; Swierczek, Sabina I.; Holz-Dygas, Anna M.; Mirkin*, Chad A.; Holz*, Richard C. "The Use of DPN to Prepare Motile Bacterial Microarrays" to be submitted.
5. Nyamjav, Dorjderem; Mirkin*, Chad A.; Liu*, Chang; Holz*, Richard C. "A Bacterial Cell Powered Biomotor" to be submitted

B. Books and/or Book Chapters

none

C. Proceedings Articles

none

6. INTERACTIONS

A. Presentations/Presentations at Meetings, Conferences, Seminars

Oral Presentations

1. AFOSR Biomimetic, Biomaterial, and Biointerfacial Scientific Program Review "Design and Development of Nanoscale Biomotor Power Units" February 3, 2003.
2. DARPA Biomotor Scientific Program Review "Design and Development of Nanoscale Biomotor Power Units" August 20, 2003.
3. University of California-Merced, Chemistry "Dinuclear Metallohydrolases: Mechanistic Enzymology to Nanotechnology " December 15, 2003.
4. Lawrence Livermore National Laboratory "Dinuclear Metallohydrolases: Mechanistic Enzymology to Nanotechnology " December 16, 2003.
5. AFOSR "Biomimetic, Biomaterial, and Biointerfacial" Scientific Program Review "Design and Development of Nanoscale Biomotor Power Units" January, 2004.
6. AFOSR Dip-Pen Nanolithography Workshop "Design and Development of Nanoscale Biomotor Power Units" January, 2004.
7. 59th American Chemical Society Northwest/18th Rocky Mountain Regional Meeting "Fabricating Microarrays of Motile Bacteria", Logan, UT, June 7, 2004.

8. AFOSR "Biomimetic, Biomaterial, and Biointerfacial" Scientific Program Review "Design and Development of Nanoscale Biomotor Power Units" January, 2005.
9. University of Oklahoma "Design and Development of Nanoscale Biomotor Power Units" February 1, 2005.
10. University of Texas, San Antonio "Design and Development of Nanoscale Biomotor Power Units" February 24, 2005.
11. North Dakota State University "Design and Development of Nanoscale Biomotor Power Units" March 2, 2005.
12. AFOSR "Biomimetic, Biomaterial, and Biointerfacial" Scientific Program Review "Design and Development of Nanoscale Biomotor Power Units" Duck Key, Florida, January4, 2006.
13. Loyola University-Chicago "Design and Development of Nanoscale Biomotor Power Units" February 21, 2006.
14. Boston University "Design and Development of Nanoscale Biomotor Power Units" Oct. 23, 2006.

Poster Presentations

1. Sergey Rozhok, Chad A. Mirkin, and Richard C. Holz "Fabrication of Bacterial Microarrays" DARPA Biomotor Scientific Review, San Francisco, CA, August, 2003.
2. Sergey Rozhok, Chad A. Mirkin, and Richard C. Holz "From Random Attachment to Single Cell Specified Arrangements" AFOSR Dip-Pen Nanolithography Workshop, Duck Kay, FL, January, 2004.
3. Sergey Rozhok, Chang Liu, Chad A. Mirkin and Richard C. Holz* "Fabricating Microarrays of Motile Bacteria" 59th American Chemical Society Northwest/18th Rocky Mountain Regional Meeting, Logan, UT, June 7, 2004.

B. Consultative/Advisory Functions

List consultative and advisory functions to other laboratories and agencies, especially Air Force and other DoD laboratories. Provide factual information about the subject matter, institutions, locations, dates, and name(s) of principal individuals involved.

None

C. Transitions

Describe cases where knowledge resulting from your effort is used, or will be used in a technology application. Transitions can be to entities in the DoD, other federal agencies, or industry. Briefly list the enabling research, the laboratory or company, and an individual in the organization who made use of your research.

None

7. NEW DISCOVERIES, INVENTIONS, OR PATENT DISCLOSURES

A. New Discoveries

B. New Inventions and/or Patent Disclosures

1. Holz, Richard C.; Mirkin, Chad A.; Liu, Chang; Rozhok, Sergey "Fabrication of Bacterial Microarrays" Provisional patent application in preparation.

8. HONORS/AWARDS

List honors and awards received during the contract period. List lifetime achievement honors such as Nobel Prize, honorary doctorates, and society fellowships prior to this effort.

Research Visiting Professor Institute for Nanotechnology and Department of Chemistry, Northwestern University, 2002-03

2/21/2007

Final Report

"Design and Development of Nanoscale Biomotor Power Units"

Grant Number: F49620-02-1-0375

PI NAME: Richard C. Holz
Utah State University
Department of Chemistry and Biochemistry
300 Old Main Hill
Logan, UT 84322

Period covered: 8/02/2003 to 11/01/2006

1. OBJECTIVES (these do not change)

1) Attachment of motile bacteria (*E. coli*, *H. pylori*, or *S. typhimurium*) in a nose-on fashion *via* biological linker molecules such as antibodies, poly-histidine, polysaccharides, polystyrene, poly-L-lysine, or oligonucleotides to gold, nickel, or glass (SiO_2) surfaces in a specific nanoarray using Dip-Pen Nanolithography (DPN). In this way, all of the bacterial cells will have full use of their flagella and all of the flagella will be oriented in the same direction, thus maximizing the mechanical energy output.

2) Determine the appropriate conditions to enhance the life of surface attached bacterial cell motility by using low levels of the antibiotic tetracycline, which prevents bacterial cell division in nutrient rich media but allows bacteria to continue to survive. Various concentrations of weak acids such as acetic acid or salicylate and the solution pH on will be investigated with respect to flagella motion. The systematic alteration of the solution surrounding the bacterial cells components will allow us to find conditions under which the motile behavior of whole bacterial cell flagella can be extended to times greater than 1 to 2 hours, thus, providing longer lived biomotors.

3) Design and build a prototype biomotor. The surface that the motile bacterial cells will be attached to will be nano-scale fins that are attached to a disk free to rotate about an output shaft. The bacterial nanoarrays on each fin will be arranged in a linear fashion so that the bacteria will be aligned in similar directions thereby maximizing the work output to push the disk. The resulting forces, which will be tangential to the disk, will rotate the output shaft and thus produce mechanical energy.